2 Neonatal Hypoxic-Ischemic Brain Insults and Their Mechanisms

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2.1 INTRODUCTION

Cerebral ischemia is an important cause of brain injury and permanent neurologic disability in fetuses and neonates, and considerable progress is being made to determine underlying causes and to develop neuroprotective interventions. In contrast to adults — where focal single-vessel occlusions from emboli or thrombi are the most common causes of cerebral ischemia — in the neonate, ischemia superimposed on severe hypoxemia secondary to disruption of ventilation or oxygen delivery is more common.[1] For example, in the syndrome of near-total asphyxia due to nearly complete disruption of delivery of oxygen to the infant through the umbilical cord, severe hypoxemia leads to reduced cardiac output, which in turn causes relatively symmetric reduction in cerebral blood flow to the brain.[2] When focal occlusion of a cerebral vessel occurs in the infant, it is more likely to be caused by infection associated with intravascular clotting, emboli of cardiac origin, or genetic clotting disorders than in adults.[3] Major differences in the selective vulnerability of brain regions also distinguish the infant from the adult. For example, selective vulnerabiltiy of the periventricular white matter in premature infants,[4] or of the basal ganglia and thalamus in term infants subjected to severe asphyxia,[5,6] are syndromes that are far less frequent in older individuals. Another factor that distinguishes the infant from the adult is the enhanced role of glutamate-mediated excitotoxicity in the infant.[7] Although the infant brain is protected from hypoxia from an energetic standpoint because of its

modest energy consumption,[8] failure to supply the neonate with a critical threshold of oxygen and glucose causes severe excitotoxic injury.[9] This injury is associated with prominent signs of heightened excitatory activity including seizures and epileptic changes on EEG and encephalopathy.[10] Developmental changes in excitatory neurotransmitter circuits, especially in the subunit composition of NMDA type glutamate receptors, may make them more likely to mediate injury in the neonate.[11] Therefore, hypoxic-ischemic insults in the neonatal brain can be distinguished from ischemic disorders in adults by their causation, age-dependent differences in regional vulnerability, and molecular factors involved in their pathogenesis.

2.2 MAJOR SYNDROMES OF NEONATAL HYPOXIC-ISCHEMIC INJURY

2.2.1 Syndromes Caused by Asphyxia

2.2.1.1 Near-Total Asphyxia

2.2.1.1.1 Pattern of Injury in Human Infants

The fetus is vulnerable to complete disruption of oxygen delivery from the placenta if the umbilical cord becomes kinked or compressed. This type of insult or absence of ventilation in neonates can result in a severe impairment of oxygen delivery together with a buildup of carbon dioxide, a condition referred to as *asphyxia*.[9] The risk of brain injury from asphyxia is most closely related to the degree of hypoxemia causing metabolic acidosis from accumulation of lactic acid. Clinical studies suggest that the risk of brain injury can reach 50% if the cord pH after an asphyxial episode falls to less than 7.0 with a concurrent metabolic acidosis associated with a base deficit of more than 20.[5,9,12,14] A recent study in asphyxiated infants showed a strong link between an elevation in the ratio of lactic acid to creatinine in urine and severity of brain dysfunction (encephalopathy).[13] Infants exposed to severe asphyxia at the end of a term gestation cannot endure the insult for longer than approximately 30 min because the heart fails to maintain cardiac output and arrests after longer intervals.[14] Infants with a brain insult associated with the syndrome of *near-total asphyxia* have undergone severe hypoxemia, acidosis, and reduced cardiac output nearly to the point of cardiac arrest but are able to be resuscitated.[2]

Neonates who have survived such an intense but relatively brief insult have a characteristic pattern of brain injury which can be visualized on MRI (magnetic resonance imaging) scanning, and sometimes on computerized tomographic (CT) radiologic scanning.[5,6,15] Within several days to weeks after the insult, an MR image weighted for T_1 (time for longitudinal magnetization relaxation) exhibits a symmetric increased signal in the peri-Rolandic sensorimotor cortex, the putamena, the thalami bilaterally, and sometimes in the brainstem.[16] Careful analysis of brain density with CT scans has demonstrated reduced density of the thalamus and basal ganglia within several days after the injury in some infants.[6] At an interval of several months to many years later, the T_1-weighted signal disappears and there is enhanced signal in the same regions on images weighted for T_2 (time for transverse magnetization

relaxation), suggesting gliosis.[5] Although very severe injury can extend beyond these selectively vulnerable regions into the central and subcortical white matter, in most infants damage from the near-total asphyxia syndrome spares completely the periventricular white matter and cerebral cortex.[2,5] This pattern of injury is virtually always associated with severe motor impairment fitting a pattern called extrapyramidal cerebral palsy.

2.2.1.1.2 Pathogenesis of the Near-Total Asphyxia Pattern

The pattern of selective vulnerability in the putamen, thalamus, and peri-Rolandic cerebral cortex corresponds to neuronal areas that are relatively more active than others in the neonatal brain but not to recognized patterns of arterial distribution.[17,18] This suggests that the pattern of injury is related more to the intrinsic vulnerability of these areas to energy failure than to regional patterns of ischemia. One potentially important link among these areas is that they are interconnected by excitatory neuronal circuits.[19] Glutamate-containing projections from the cortex innervate both the thalamus and putamen, which is the primary motor component of the basal ganglia. The thalamus in turn sends excitatory projections to the sensorimotor cortex. Overactivity in these excitatory pathways could contribute to excitotoxic injury in these regions, and clinical evidence of excessive excitatory activity after an asphyxial insult is consistent with this hypothesis.[20] Functional brain imaging using positron emission tomography (PET) with ^{18}Fluro-deoxyglucose (FD-glucose) in human infants indicates that these selectively vulnerable regions are also relatively more active than other regions in the period after an asphyxial insult.[17,18] Experimental evidence indicates that the regional cerebral metabolism of glucose (rCMRgl) in the brain is tightly linked to the turnover of glutamate at synapses, suggesting that the relative increase in rCMRgl observed after asphyxia may correlate with increased activity at excitatory synapses.[21] Therefore, the selective pattern of injury seen after near-total asphyxia in human neonates may reflect the location of vulnerable regions within maturing excitatory circuits.[22]

2.2.1.1.3 Models of Near-Total Asphyxia

Several models of near-total asphyxial brain injury in human neonates have been developed in laboratory animals, including subhuman primates and piglets.[14,23] Although there are important differences between the models and the human disorder, there are also remarkable similarities, especially with respect to the pattern of selective neuronal vulnerability.

2.2.1.1.3.1 Acute Total Asphyxia in Monkeys

Myers' model of acute total asphyxia in monkeys remains one of the best replications of the insult seen in human neonates. In this model, term monkey fetuses were exposed to timed intervals of 10 to 25 min of asphyxia by occluding the umbilical cord and slipping a thin, saline-filled rubber sac over the fetal head at surgical delivery.[14] The neonates were resuscitated with ventilation, cardiac massage, and intra-arterial epinephrine and maintained for intervals of several days to weeks after the insult. The initial insult was associated with a marked drop in systemic blood pressure to below 10 mm Hg, severe metabolic acidosis with a pH as low as 6.7, and a base

deficit approaching 20 mEQ. In this model the first evidence of brain injury one week later was observed after 10 minutes of asphyxia, while animals asphyxiated for longer than 25 minutes generally died of heart failure shortly after the insult.

Neuropathologic examination of the brains in these animals demonstrated widespread injury in the thalamus and brainstem when the insult lasted longer than 16 to 18 min.[14] Myers noted that the most vulnerable structures, such as the posterior and lateral ventral thalamic nuclei, also had the highest regional cerebral blood flow in the newborn period in the baseline state when studied with ^{14}C-antipyrine autoradiography.[14] This is consistent with the hypothesis that vulnerable structures have a relatively high baseline level of neuronal activity. Interestingly, Myers commented that the injury pattern produced in the monkey fetus bore no relation to human perinatal damage recognized at the time. He speculated that this was based in part on the fact that the newborn monkey is more mature than the human term neonate. However, modern MR imaging does reveal that the thalamus is a focus of injury in most infants with the syndrome of near-total asphyxia.[5,6]

2.2.1.1.3.2 Acute Asphyxia in Piglets

Asphyxia in one-week-old piglets has provided one of the best models for the neuropathologic and neuroimaging pattern of injury observed in human neonates with the syndrome of near-total asphyxia.[23] In the model described by Martin et al.,[23] one-week-old piglets are intubated under anesthesia with pentobarbital, paralyzed, and ventilated, and physiologic variables and temperature are controlled. Hypoxemia is initiated by reducing the arterial oxygen saturation to 30% for 30 min, followed by 5 min of ventilation with room air, followed by 7 min of airway occlusion during which arterial oxygen saturation is reduced to less than 5%. At the end of asphyxia, the animals are resuscitated with 100% oxygen and chest compressions, and epinephrine and bicarbonate are infused to maintain arterial pH at 7.4. Animals that survive 4 d are perfusion-fixed for pathologic examination.

Analysis of six piglets prepared in this way revealed a selective pattern of injury in the somatosensory cortex, basal ganglia (particularly in putamen, subthalamic nucleus, and substantia nigra), and ventral thalamus that strikingly resembles the pattern seen in neuroimaging in human neonates with near-total asphyxia.[5,6] It is noteworthy that in animals with less severe injuries, there were lesions in the thalamus, putamen, and small areas of somatosensory cortex, while more severely injured animals had involvement of the caudate and more extensive involvement of the cerebral cortex. The preferential involvement of the thalamus and putamen parallels lesions seen on MR images in human neonates.

Thoresen et al. have also developed a useful model of asphyxial injury in piglets in which the degree of hypoxemia is controlled by monitoring the amplitude of EEG activity.[24] This model has been extensively studied from the standpoint of the relationship between encephalopathy and neuropathology. In this model, one-day-old piglets are intubated and ventilated under halothane anesthesia, and physiologic and EEG monitoring are initiated. After a period of stabilization, the concentration of inspired oxygen (FiO_2) is abruptly reduced to 6% and maintained at the highest value at which the EEG amplitude is reduced to 7 μV or less. Blood pressure in these

animals ranged from 23–40 mm Hg, and animals that arrested were resuscitated with 100% oxygen and cardiac compressions. Groups of animals were compared with sham, short (mean of 27 min) and long (mean of 40 min) insults.

In an analysis of 19 animals prepared in this way, Thoresen et al.[24] recorded post-insult seizures that resembled those in human neonates with asphyxia in 39% of the animals. The insult produced extensive neuropathologic damage that preferentially affected the cortex, hippocampus, and cerebellum, more than the thalamus and basal ganglia, suggesting many of the animals prepared in this way sustain more of a partial-prolonged insult, as described below, than the human syndrome of near-total asphyxia. This model has been very useful to study the pathogenesis of hypoxic-ischemic encephalopathy and postasphyxial seizures seen in human infants.

2.2.1.2 Partial Prolonged Asphyxia

2.2.1.2.1 Injury in Human Infants

In many infants, the asphyxial insult is not abrupt and extremely severe as in the near-total syndrome. Rather, it is sustained over a period of several hours.[14, 25] The major physiologic difference between the near-total and partial prolonged insults is that cardiac pumping is maintained in the latter, while cardiac arrest terminates the near-total insult after 30 min to 1 h.[14] Although there are instances of overlap between these two types of insult in clinical practice, in many cases the patterns seen on neuroimaging and neuropathology are remarkably distinct. In contrast to the triad of putamenal, thalamic, and somatosensory cortical pathology seen after near-total asphyxia, partial prolonged insults generally produce predominantly cortical involvement.[12] In the period of several days immediately following the insult, there is generally extensive cortical edema and blurring of the gray-white matter junction seen on neuroimages. After several months, the cortex has often been replaced by multiple cysts, the pattern of multicystic encephalomalacia.

2.2.1.2.2 Experimental Models

2.2.1.2.2.1 Partial Prolonged Asphyxia in Monkeys

Myers produced partial asphyxia in monkey fetuses by constriction of the maternal aorta, which lowers the fetal arterial partial pressure of oxygen and pH in a stepwise fashion depending on the aortic blood pressure.[14] At levels of blood pressure that produced a pH of 7.10 or 7.15, no brain injury occurred despite a duration of several hours. However, fetal brain injury did occur with acidosis below pH 7.0, with partial asphyxia lasting from a half hour to several hours. Myers was able to produce a spectrum of neuropathology in these animals ranging from virtually complete cortical hemispheric necrosis with little basal ganglia injury with partial asphyxia lasting several hours to combinations of basal ganglia and severe cortical injury when episodes of total asphyxia were superimposed on milder more prolonged episodes.[14] These changes resemble those seen in human neonates with similar insults.

2.2.1.2.2.2 Partial Prolonged Asphyxia in Piglets and Rodents

Several other animal models replicate some features of partial prolonged asphyxia as seen in human infants. As mentioned above, Thoresen's piglet model produces a predominantly cortical and hippocampal neuropathology that resembles partial asphyxia in monkeys and human infants.[24] Other piglet models have produced partial asphyxia by combining hypoxia with periods of carotid occlusion and hypotension or by inflating a cuff around the neck at pressures above systemic blood pressure.[26-28] These preparations are different from models described above because they do not expose the heart and other organs to asphyxia as occurs in human neonates. In another acute, nonsurvival model partial prolonged asphyxia is induced by ventilating piglets with 5–7% oxygen for 60 min.[29]

Vannucci's laboratory pioneered the combination of unilateral carotid ligation and exposure to 8% oxygen to produce hypoxic-ischemic injury in 7-day-old rats, a model that has been used extensively in physiologic, neurochemical, and neuroprotection studies.[30] The model replicates certain features of injury in human neonates, especially the relatively selective vulnerability of the basal ganglia (corpus striatum in the rodent), cerebral cortex, and thalamus. It also appears to replicate the fact that brain injury is dependent on reduction in cardiac output. Unlike the adult Levine preparation, which it resembles, studies of regional cerebral blood flow showed that cerebral perfusion on the side of carotid ligation is normal in the baseline state but falls by 70% or more during the period of hypoxia.[1] Therefore, the insult resembles asphyxia in the human infant, in which ischemia is superimposed on severe hypoxemia. After recovery, perfusion on the ligated side is restored, in contrast to focal occlusion models. Neuronal injury in this model is strongly dependent on NMDA-mediated glutamate neurotoxicity.[7] This is a convenient model with many strengths, and one which combines several features of both partial prolonged and near-total asphyxia. Weaknesses in the model include the difficulty in controlling physiologic variables, as in larger models, and a relatively high rate of interanimal variability in brain injury.[31] A similar model of common carotid artery ligation and exposure to hypoxia in the rabbit has been reported by D'Arceuil et al.[32]

2.2.1.3 Parasagittal Watershed Infarction

2.2.1.3.1 Injury in Human Infants

The parasagittal watershed infarction syndrome in human neonates following asphyxia was described by Volpe and Pasternak using clinical criteria and technetium-enhanced brain scanning in an infant after asphyxia associated with severe systemic hypotension.[33] Infants with this syndrome typically have weakness in the upper arms and shoulders compared to the hands and lower extremities due to injury to cerebral cortex and subcortical white matter in the vascular watershed between the anterior and middle cerebral arteries. Severe loss of blood pressure causes diminished perfusion and infarctions in zones that are midway between end-vessels served by these two major cerebral vessels. This syndrome has been documented by postitron emission tomography (PET) as well as by conventional neuroimaging.[34]

2.2.1.3.2 Experimental Models of Parasagittal Watershed Infarction

Myers produced parasagittal watershed infarctions in newborn monkeys with severe hypotension superimposed on partial prolonged asphyxia[14] and Pasternak and Groothuis produced similar lesions with hypotension in newborn puppies.[35] More recently, Williams and colleagues[36] produced a model of parasagittal infarction in fetal sheep. In this preparation the head, neck, and forelimbs of fetal sheep of 119–126 d gestation are externalized from the uterus and the vertebro-occipital anastomoses between the carotid arteries and veretebral arteries are ligated bilaterally. This procedure restricts the vertebral vascular supply to the carotid arteries. Inflatable occluder cuffs are placed around the carotid arteries and shielded electrodes are inserted via parasagittal burr holes overlying the parietal lobe of the brain. To produce cerebral ischemia, the carotid artery cuffs are inflated for 10–40 min and after which animals survive for 3 d. In this model, the greatest damage occurs in the parasagittal cortex and CA1 and CA3 regions of the hippocampus, with much less injury in the lateral cortex, striatum and thalamus. In contrast to the human, in which carotid occlusion would allow blood flow to be redistributed through the circle of Willis from the posterior circulation, in this model in the sheep, the pressure in the entire anterior circulation of the brain can be controlled by flow in the carotid arteries. The model has been used to advantage to study the evolution of seizures following neonatal asphyxia. Intrauterine electrocortigraphic monitoring demonstrated that onset of seizures in the model is delayed approximately 8 h after asphyxia, similar to the time period for appearance of seizures in humans following asphyxia.

2.2.1.4 Syndrome of Hypoxic-Ischemic Encephalopathy (HIE)

Each of these patterns of asphyxial injury in human full-term neonates is associated with the clinical syndrome of hypoxic-ischemic encephalopathy (HIE).[10] HIE describes clinical manifestations of brain dysfunction including seizures, decreased level of consciousness, and hypotonia as well as electroencephalograhic abnormalities such as electrographic seizures, a burst-suppression pattern and/or severe slowing or voltage suppression.[9,37] Signs of HIE in human neonates usually evolve after a delay of 8–24 h after the asphyxial insult, then worsen and improve over the next week to 10 d.[9,38] Clinical studies suggest that the stage and severity of clinical HIE based on clinical and EEG manifestations is directly related to the severity of brain injury.[39] Signs of mild encephalopathy on the Sarnat scale, which include autonomic hyperactivity and hyperalertness and mild EEG changes, generally are not associated with permanent brain injury while severe encephalopathy usually is associated with permanent injury as described above. Infants who have had an asphyxial insult, but do not manifest clinical signs of HIE during the following week of observation in the nursery, have virtually no chance of sustaining a permanent brain injury.[40] This makes assessment of the clinical and EEG signs of HIE important for predicting which infants are at greatest risk for permanent brain injury.

There appears to be an important physiologic principle underlying the observation that evolving injury from asphyxia is reflected in the severity of clinical signs of HIE in neonates. The infant is generally more likely than the more mature brain to respond to a variety of insults and injuries with excitatory signs such as seizures and epileptic EEG patterns than the older child or adult.[41] For example, approximately 10% of young children experience seizures with high fever without any underlying neuropathology but this is very uncommon at later ages. Seizures of all types are more common in infants and young children than in older individuals as are the EEG abnormalities associated with them.[41] This may be related in part to the fact that excitatory synapses are rapidly proliferating in the immature brain, reaching levels approximately twice as high as the adult at age 2 years in humans.[42] Glutamate-mediated excitatory neurotransmission probably contributes to the enhanced excitatory activity in the neonate.[7] For example, the physiologic activity of the N-methyl-D-aspartate (NMDA)-type glutamate receptor appears to be enhanced in the immature brain and may contribute to seizures as well as neuronal damage.[41] Excitatory signs of brain dysfunction in HIE probably correlate strongly with the degree of brain injury from asphyxia because the neonatal brain is intrinsically more excitable than the adult and because, as described in more detail below, dysfunction of excitatory synapses contributes directly to the evolution of the injury.

2.2.2 Syndromes Caused Primarily by Ischemia

2.2.2.1 Focal Ischemic Infarction

2.2.2.1.1 Injury in Human Neonates

Brain injury caused by focal ischemic infarctions is less common than asphyxial syndromes in neonates and they appear to be caused by fundamentally different mechanisms.[3] Except for rare situations, such as in cases of carotid cannulation of the carotid artery used for extracorporeal membrane oxygenation (ECMO) therapy in very hypoxic and hypotensive infants, it is unusual for asphyxia to be associated with single vessel strokes.[43] Cerebral infarcts in fetuses and neonates are most likely to be caused by arterial emboli, and the most common cause of these emboli is infection related to sepsis and disseminated intravascular coagulation.[3] Emboli from intracardiac sources are becoming less common as early repair of cyanotic congenital heart disease has become commonplace. Inherited disorders of coagulation including Leiden Factor V mutation, antiphospholipid antibodies, homocystinuria and protein C, and protein S deficiencies are gaining wider recognition as causes of neonatal strokes.[44,45] It is also noteworthy that many of these causes of increased coagulability are also also associated with an increased risk of complications of pregnancy in the mother such as pre-eclampsia and toxemia.[46] Other genetic syndromes, such as carbohydrate-deficient glycoprotein disorder, have also been recognized relatively recently as carrying a higher risk of stroke.[47] This information suggests that focal ischemic infarctions in neonates are generally caused by different mechanisms than the asphyxia syndromes discussed above. Although many focal infarctions are recognized at birth by the onset of focal seizures, epidemiologic information suggests

that more strokes associated with permanent hemiparetic cerebral palsy probably originate prenatally than in the immediate intrapartum period.[48]

Much less is known about the mechanisms for infarctions on the venous side of the circulation which are responsible for two additional neonatal disorders: venous sinus thrombosis and periventricular hemorrhagic infarction. Venous sinus thrombosis is being recognized more frequently with modern neuroimaging including color Doppler ultrasound of the brain and MR imaging and MR angiography.[9,49] Although no large series have been reported, infection, disseminated intravascular coagulation, dehydration, and thrombocytosis secondary to acquired disorders such as polycythemia and inherited clotting disorders are potential causes.[9] Thrombosis of the internal cerebral vein with rupture and intracranial hemorrhage, called periventricular hemorrhagic infarction, has also been implicated in the pathogenesis of grade III-IV intracranial hemorrhage in premature infants.[4]

2.2.2.1.2 Focal Ischemia Models

Focal cerebral ischemia has been studied less commonly in neonatal experimental models of focal infarction than asphyxia models. Ashwal and colleagues have used an 18-day-old rat model in which the middle cerebral artery is occluded transiently by passing a 0.07-mm nylon filament into it via the carotid artery.[50] This preparation produces a relatively reproducible area of injury, making it possible to distinguish core from penumbral regions, and has been used to study the neuroprotective effects of nitric oxide synthase inhibition.[51] Renolleau et al. also developed a focal occlusion model in 7-day-old rat pups by permanently occluding the left middle cerebral artery in association with a one-hour period of reversible occlusion of the left common carotid artery.[52] A similar model has also been reported in 7-day-old rats by Derugin, et al.[53]

2.2.2.2 Periventricular Leukomalacia (PVL)

Periventricular leukomalacia or PVL is one of the most important causes of disability in premature infants and is the most common cause of permanent motor dysfunction or cerebral palsy in this group.[4] PVL is generally considered as a developmentally determined ischemic syndrome of premature infants with a peak incidence between[27–30] weeks gestation.[4] However, as clinical study of the disorder has intensified, etiologies such as maternal and neonatal infection have also emerged as important causes.[54] Nevertheless, it is appropriate to condsider PVL here as an important cause of ischemic injury in the developing brain.

PVL was originally described in postmortem brain by Banker and Larroche as symmetric bilateral necrosis of periventricular white matter that is most severe around the frontal horns and in the occipital-parietal region around the ventricular trigone.[55] As neuroimaging with head ultrasound and then MRI scanning developed, PVL could be studied in more detail in living infants and a greater variety of lesions was apparent.[56] PVL associated with cystic lesions in the white matter can often be observed to develop in premature infants with serial imaging in the postnatal period.[56] In this type, there may be little evidence of pathology initially after birth, but

serial ultrasound images first reveal enhanced echogenicity around the ventricles and then formation of cysts.[57] These cysts eventually collapse after several weeks, as the surrounding white matter reorganizes, simultaneously enlarging the ventricular space filled by cerebrospinal fluid. In many cases, the formation of these periventricular cysts is probably caused by ischemia in the periventricular white matter, which may be more vulnerable because of the anatomy of its arterial supply.[58] Although the role of systemic hypotension has been proposed as a cause of PVL, this theory currently appears to have less support than the hypothesis of impaired intracerebral arterial autoregulation.[59] Premature infants cannot regulate the diameter of intracerebal arterioles to maintain constant perfusion pressure in the face of variations in systemic pressure as well as older babies. Careful studies with Doppler ultrasound suggest that there is a strong correlation between fluctuations in cerebral perfusion in sick premature infants and development of PVL.[60]

Ischemia may cause white matter necrosis through excitotoxic mechanims that parallel those involved in neuronal damage.[61] Immature oligodendroglia that synthesize and maintain myelin are vulnerable to high concentrations of glutamate that may be released in white matter during ischemia, in part because they are more vulnerable to damage from oxidative stress.[62] The enhanced vulnerability of immature oligodendroglia during a critical point in their development has been proposed as one of the factors that makes premature infants most vulnerable to PVL during a relatively narrow time window of gestational age.[63] Excitotoxic mechanisms may also be a link to death of oligodendroglia from inflammatory cytokines stimulated by infection.[54] Both agents such as endotoxin and overstimulation of excitatory amino acids may stimulate proinflammatory cascades that kill oligodendroglia by apoptotic mechanism. This mechanistic interaction between glutamate-mediated excitotoxicty stimulated by ischemia and inflammatory cascades stimulated by infectious agents, as well as the association between infection and alterations in blood pressure, probably contribute to the interactions between these two mechanisms to cause PVL.[54]

2.2.2.3 Hypothermic Circulatory Arrest With Heart Surgery

2.2.2.3.1 Insult in Human Neonates

Hypothermic circulatory arrest (HCA), in which total circulatory arrest is combined with reduction in body temperature of 18°C or lower, is commonly used to support surgery for complex congenital heart disorders such as transposition of the great vessels. This procedure is associated with brain injury in a small group of childern, who may have severe permanent impairment.[64] One of the most severe disorders associated with HCA is the syndrome of postpump choreoathetosis related to injury to the basal ganglia.[65] This syndrome typically begins after a latent period between the time when the infant awakes from anesthesia and onset of reduced level of consciousness, agitation, and choreoathetotic movements. Kupsky and colleagues found that this syndrome was associated with damage to the globus pallidus in postmortem analysis of 2 patients.[66] Holden et al. identified basal ganglia lesions on neuroimaging in 6 of 11 patients with postpump choreoathetosis and identified cyanosis as a risk

factor.[65] Curless et al. reported that severe respiratory alkalosis and hypocapnia during rewarming might be a risk factor in 3 patients they reported with postpump choreoathetosis.[67] Respiratory alkalosis occurs in patients maintained by the so-called "alpha-stat" method of acid base management during heart surgery. Du Plessis et al. reported that use of the more acidotic "pH-stat" strategy was associated with a better neurologic outcome in a study of 182 infants undergoing deep hypothermic cardiopulmonary bypass.[68] This suggests that severe respiratory alkalosis may worsen ischemia during reperfusion.

HCA may also cause seizures and cognitive impairments in some infants. Newburger et al. compared HCA with low-flow cardiopulmonary bypass in infants undergoing heart surgery for D-transposition of the great vessels and found that HCA increased the odds ratio for postoperative seizures to 11.4 and lengthened the recovery time to the first appearance of EEG activity.[69] Bellinger et al. analyzed the developmental and neurologic status on the same group of infants and found that the HCA group had a higher percentage of low developmental scores (27% vs. 12%).[70] Developmental scores in the HCA group were inversely related to the duration of circulatory arrest and the incidence of neurologic abnormalities were positively related to the length of arrest. These studies comparing two methods of support for cardiac surgery, rather than simply comparing HCA with control children, are important because the rate of neurobehavioral disorders in infants with congenital heart disease prior to surgery is elevated.[71] A prolonged period of severe cyanosis prior to surgery has also been identified as a risk factor for cognitive decline in children with transposition of the great arteries by Newburger et al.[72]

2.2.2.3.2 Models of Brain Injury from Hypothermic Circulatory Arrest

Several groups have developed experimental models of hypothermic circulatory arrest in dogs and piglets. Vannucci's group developed a model of hypothermic circulatory arrest for 1 to 1.75 hours in newborn dogs induced by intravenous potassium chloride followed by resuscitation with closed chest compression, epinephrine, and bicarbonate administration with a recovery period up to 72 hours.[73] They found neuropathological evidence of damage in cortex and basal ganglia that was related to the duration of arrest and a relationship between the severity of cortical injury and neurobehavioral abnormalities.

Nomura et al. developed a model of hypothermic circulatory arrest and cardiopulmonary bypass in one-week-old pigs that replicates more closely the clinical approach used in heart surgery.[74] They used near-infrared spectroscopy (NIRS) to examine intravascular hemoglobin and mitochondrial (cytochrome aa_3) oxygenation and found changes similar to that reported in human infants by du Plessis et al.[75] Although cooling was associated with an increase in hemoglobin oxygenation despite the reduction in cerebral blood flow, cytochrome aa_3 continued to fall during cooling, consistent with a net cellular oxygen deficit. In human infants, reperfusion following circulatory arrest caused a rapid return of intravascular hemoglobin oxygenation but the level of oxidized cytochrome aa_3 was delayed, especially in infants over 2 weeks of age.[75] Recent studies in the piglet model indicate that measurement

of a reduction in cytochrome signal on NIRS correlates well with concurrent reductions in high-energy phosphates and evidence of histologic brain injury.[76] Therefore, work in this piglet model suggests that NIRS might be a useful clinical tool for monitoring brain oxygenation during HCA.

Another model of hypothermic circulatory arrest and cardiopulmonary bypass in older dogs has allowed extensive studies of mechanisms of glutamate-mediated excitotoxcity and neuronal death that are relevant to injury in neonates.[77] In this model, 10-month-old hound dogs are placed on cardiopulmonary bypass under anesthesia and cooled to 18°C over 30 minutes. Then the pump is turned off and venous blood is drained and circulatory arrest is maintained for 2 hours. After circulation is restored, animals are rewarmed and maintained for intervals up to 72 hours. Circulatory arrest results in selective neuronal necrosis and apoptosis in layers 3 and 5 of entorhinal cortex and neocortex, and in the basal ganglia, Purkinje cells of the cerebellum, and CA-1 of the hippocampus. Pretreatment with the NMDA antagonist MK-801[77] and the neuronal nitric oxide synthase (nNOS) inhibitors 7-nitroindazole and AR17477 (Astra Arcus) are strongly protective against histopathologic and neurologic manifestations of injury.[78] Studies utilizing microdialysis indicate that extracellular levels of glutamate and citrulline, which reflect the production of nitric oxide from arginine, are markedly elevated in the period immediately after restoration of circulation.[78] Immunocytochemical studies indicate that immunoreactivity for nNOS containing neuronal fibers is also induced over 24 hours after the insult.[79] These studies suggest that the syndrome of delayed onset of encephalopathy, seizures, and movement disorders after hypothermic circulatory arrest reflects a prolonged elevation in extracellular glutamate and activation of excitatory amino acid receptors as well as induction of nNOS and prolonged elevation in nitric oxide (NO) during the reperfusion period.

2.3 MECHANISMS FOR DELAYED NEURONAL INJURY

The neonatal brain has very low energy requirements,[8] and reactive hyperemia in response to even severe hypoxemia can usually maintain levels of ATP (adenosine triphosphate) through anaerobic glycolysis. However, failure of cerebral perfusion superimposed on severe hypoxemia allows ATP to fall to critical levels,[1] triggering the first steps in a cascade of biochemical events that can result in death of brain tissue.

2.3.1 Excitotoxic Mechanisms

One of the earliest events triggered by the initial phase of energy failure is depolarization of neuronal membranes and disruption in synaptic function. The prominent role that excitatory neuronal activity plays in the clinical signs of encephalopathy that evolve after hypoxic ischemia[10] appears to reflect the relatively selective impact of the insult on excitatory synapses and excitatory neuronal circuits. Disruption in pre- and postsynaptic components of synapses and persistent, enhanced firing in these neuronal circuits probably contribute to selective patterns of brain injury.

2.3.1.1 Disruption of Glutamate Synapse Function

Hypoxic ischemia severe enough to damage the neonatal brain produces a prominent disruption in the function of glutamate synapses by reducing the reuptake of glutamate into glia by its energy and sodium-dependent transporter.[80–82] This leads to accumulation of neurotransmitter within synapses and the extracellular space.[83,84] In human infants after asphyxia, levels of glutamate, aspartate, and glycine are elevated in cerebrospinal fluid in proportion to the severity of HIE.[85,86] Disruption of glucose delivery to ischemic tissue may be responsible for early reduction in the activity of glutamate transporters because, in the face of hypoxemia, increased glucose is needed to produce ATP anaerobically to power Na^+/K^+ ATPase linked to the transporter.[21]

Postsynaptic glutamate receptors are activated through combinations of elevated neurotransmitter levels and depolarization of postsynaptic membranes.[87] NMDA (N-methyl-D-aspartate) glutamate receptor-operated channels and voltage-sensitive calcium channels open when mitochondrial energy levels fall.[88] Experimental evidence suggests that NMDA receptors are strongly activated during hypoxic ischemia, through a combination of elevated levels of glutamate and glycine in the synapse and depolarization of neuronal membranes from energy failure with passive opening of NMDA receptors.[87,89,95] This allows potentially toxic levels of intracellular calcium and sodium to enter neurons and some glia, including oligodendroglia.[61] NMDA receptor antagonist drugs are strongly neuroprotective in neonatal models of hypoxic ischemia, reflecting the fact that damage mediated by the NMDA receptor is markedly enhanced in the immature brain compared to the adult.[90–94] Therefore, combinations of hypoxemia and ischemia have a profound effect on pre- and postsynaptic elements of excitatory synapses.

2.3.1.2 Selective Vulnerabilty Due to Connectivity of Neuronal Circuits

As described in the section on near-total asphyxia pattern, certain groups of neurons, such as those in the thalamus, putamen, and sensorimotor cortex, may be predisposed to injury by virtue of their location within excitatory neuronal networks.[22] The elevated rCMRgl in vulnerable regions in the period after the insult may reflect elevated levels of glutamate release and reuptake in excitatory projections from the sensorimotor cortex to the putamen and reciprocal connections between the cortex and the thalamus that could mediate excitotoxic injury.[22] These regions may be selectively vulnerable to near-total asphyxia because of their connectivity through excitatory pathways.[22]

2.3.2 The Neurotoxic Cascade and Delayed Energy Failure

Hypoxic ischemia and disruption of synaptic function trigger the opening of membrane channels that allow calcium and sodium to flood into neurons and some glia, such as oligodendroglia, and these ionic changes trigger a cascade of intracellular events that can result in cell death (Figure 2.1). One of the early events in this cascade

is the accumulation of free radicals of oxygen including superoxide ($\cdot O^-$) and hydroxyl ions ($\cdot OH^-$), as well as the free radical gas, nitric oxide (NO).[1,96] Free radicals of oxygen are generated after hypoxic ischemia due to defects in the electron transport chain caused by calcium overload of mitochondrial and inadequate saturation of cytochrome oxidase with oxygen, as well as by enhanced synthesis of prostaglandins and the conversion of hypoxanthine to xanthine and uric acid.[98] Nitric oxide is produced in the postinsult period by neuronal nitric oxide synthase (nNOS) or immunologic NOS (iNOS). Transgenic mice lacking the gene for nNOS (NOS I) are protected from hypoxic ischemic injury compared with controls who express the gene, suggesting that nitric oxide stimulated by NMDA-mediated activation of nNOS contributes to brain injury.[99] Enhanced production of $NO^{\bullet}$ has been documented in several models of HIE.[78,100,101] Individual oxygen free radicals or combinations with nitric oxide, such as the combination of nitric oxide with superoxide to form peroxynitrite, can attack attack lipid membranes.[98,102] Nitric oxide can also impair metabolic steps involved in energy metabolism including glycolysis, the tricarboxylic acid cycle, and the mitochondrial electron transport chain.[98] Attack on DNA by oxygen free radicals or NO can also trigger the DNA repair enzyme poly(ADP-ribose) polymerasae (PARP), with polyadenylates nuclear proteins as part of the repair process.[102,103] Activation of PARP consumes nicotinamide adenine dinucleotide (NAD^+), an intermediate that is needed for mitochondrial production of ATP, further impairing mitochondrial function. Activation of PARP has been linked to overstimulation of NMDA, but not non-NMDA glutamate receptors.[104] DNA fragmentation produced by cysteine proteases (caspases) also contributes to activation of PARP. These steps play an important role in delayed energy failure after hypoxic ischemia.

Neuroimaging studies of infants after hypoxic-ichemic insults as well as histologic study of experimental models of ischemic injury indicate that damage evolves over a period of several days.[105] Magnetic resonance spectroscopy has been used to measure high-energy phosphorus intermediates as well as lactate in human infants after hypoxic ischemic insults.[106] These studies revealed that full-term infants had normal levels of high-energy phosphates soon after resuscitation from asphyxia, but levels dropped in infants with severe injury after 24 hours.[106] Studies with proton spectroscopy showed that infants with elevated levels of lactate/N-acetylaspartate had poor neurologic outcome.[107] Similar spectroscopic changes showing a delay in energy failure that corresponds with poor outcome have been reproduced in a piglet model of asphyxia.[108] In the 7-day-old rat pup model of hypoxic ischemia, impairment of mitochondrial function following the insult has been shown to be related in part to overstimulation of NMDA recetpors.[109] These results are consistent with the concept of hypoxic ischemia as a cascade of delayed biochemical events that impair mitochondrial oxidative metabolism over a period of hours to days after the initial insult.

2.3.3 The Apoptosis-Necrosis Continuum

Delayed or secondary energy failure causes cell death either by the genetically programmed process of apoptosis or by the explosive destruction of cellular membranes

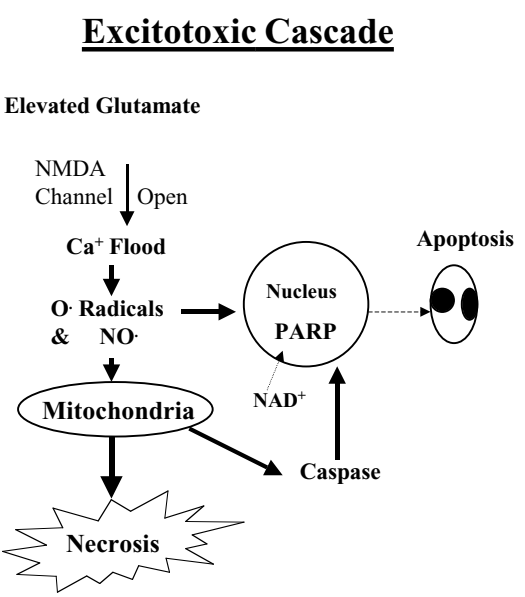

FIGURE 2.1 Schematic of the cascade of major events that lead to cell death in the neonatal brain after an hypoxic-ischemic insult.

called necrosis (Figure 2.1)[110,111]. Although a great deal of work remains to be done in specific models of ischemia in neonates, a general conceptual outline is available based on both animal and cell culture experiments.[112] Apoptosis appears to be far more prominent in neonatal animal models of hypoxic ischemia than in the adult, and cell death is expressed in a continuum from apoptosis to necrosis at this age.[113–117] In neonates it is common to observe "hybrid" cells that have morphologic features of both apoptosis and necrosis.[118,119] The same continuum is observed in the immature brain when excitotoxic amino acids are injected directly into the brain.[118] Evidence that a pan-caspase inhibitor is strongly protective against hypoxic-ischemic damage in the 7-day-old rat model suggests that apoptosis plays a major role in cell death at this age.[120,121] In the neonatal rat model of hypoxic ischemia, NMDA antagonists prevent activation of caspase 3 in the brain.[122] In the same model, apoptosis persists for more than a week after the insult, suggesting that new cells continue to commit to apoptosis over that time.[119] Postmortem neuropathology from neonates who have died after hypoxic ischemia also demonstrates prominent apoptosis.[113] This may be related to the fact that cellular programs for apoptosis are more active in the immature brain because they are used normally to remove redundant neurons.

Regarding Figure 2.1, synaptic dysfunction involving accumulation of glutamate and other amino acids and depolarization of neuronal membranes leads to excessive calcium entry into neurons and some glia. Production of oxygen-free radicals leads to mitochondrial dysfunction and secondary energy failure over hours to days after

the insult. Consumption of NAD^+ by activation of PARP poly(ADP-ribose) polymerase may contribute to mitochondrial dysfunction by reducing energy intermediates. Activation of caspases by mitochondrial dysfunction can lead to apoptosis. The nature and intensity of mitochondrial dysfunction may help to determine whether neurons die by necrosis, with total destruction of neuronal membranes, or apoptosis, which involves nuclear condensation and cytoplasmic shrinkage with membranes left intact until phagocytosis.

Experiments in cell culture suggests that the severity of mitochondrial energy failure may have a connection to the decision that cells make for apoptosis or necrosis.[110,111,123] Ankarcrona et al. found that the expression of apoptosis or necrosis in cultured neurons was related to the severity of energy failure produced by NMDA, with necrosis associated with more intense excitotoxic insults.[110] Less severe insults may impair metabolism and stimulate mitochondria to release cytochrome C and other proteins to activate caspase 3 and apoptosis by the intrinsic pathway.[124] Recent evidence suggests that hypoxic-ischemic injury in neonatal rats is also associated with activation of the extrinsic Fas death receptor pathway associated with cleavage of procaspase 8 and downstream activation of caspase 3.[125] Activation of other proteases such as the interleukin 1 β converting enzyme (ICE) family also appear to be important in triggering apoptosis after hypoxic ischemia injury in neonates, providing a connection to cytokine-mediated inflammatory pathways.[126,127] The calpain protease system is also activated by hypoxia-ischemia.[126] This information suggests ways in which the nature and severity of energy disorders in the neonatal brain influence the decision for apoptosis or necrosis.

2.3.4 Experimental Interventions to Modify Injury

Numerous interventions have been shown to reduce brain injury in experimental neonatal models although most are effective only when given before the insult or within a few hours afterward.[96,129] So far it has been difficult to translate these observations into useful clinical therapies, in part because of toxic side effects and in part because of the difficulty of chosing comparable groups of patients for clinical trials.

2.3.4.1 Hypothermia

Hypothermia is clearly protective against brain injury from hypoxic ischemia when started before the insult, as demonstrated by its use since the 1950s to protect the brain during infant cardiac surgery.[130] Several studies in the 1950s also suggested that hypothermia might be neuroprotective for asphyxiated infants, but the practice was abandoned a short time later when it was reported that premature infants had a higher mortality if exposed to hypothermia.[130] Recently there has been a resurgence of interest in this form of therapy, following on reports from several animal models that mild hypothermia reduces falls in ATP, rises in brain lactate, elevations in nitric oxide production, and brain injury associated with asphyxia.[131,101,132] Results from these experimental models suggest that the duration of postinsult hypothermia is important since short periods can delay but ultimately not prevent brain injury.[133,134] Mild

selective cooling of the head is being examined in human infants and experimental models with potentially positive results.[135] In the near-term fetal sheep model of transient cerebral ischemia, delayed selective head cooling begun before the onset of postischemic seizures and continued for 3 days was associated with improvement in EEG activity and a reduction in neuronal loss in the cortex.[136] There remain questions about whether head cooling can penetrate into deep brain regions, which are vulnerable to ischemic injury.[137] Although the method was effective in cooling deep regions of the piglet brain, numerical modeling of temperature distributions within the neonatal human head suggest that deep cooling may require a combination of systemic and head cooling.[137,138] No adverse effects were noted in a safety study in 22 term infants with asphyxia exposed to combinations of mild systemic and head cooling.[135] Even if it is not effective alone, mild hypothermia may prolong the therapeutic window in which other interventions, such as glutamate antagonists, could be effective.

2.3.4.2 Oxygen Free Radical Scavengers

Scavengers of cytotoxic oxygen free radicals include the xanthine oxidase inhibitor allopurinol, the cyclooxygenase inhibitor indomethacin, and the iron chelator desferoxamine which can prevent iron from reacting with oxygen free radicals to form more toxic intermediates.[96] Several studies in the neonatal rat model of hypoxic ischemia have demonstrated that high doses of allopurinol prevent brain injury, probably by a direct free radical scavenging mechanism rather than by inhibiting xanthine oxidase.[140] Shadid et al. showed that allopurinol, indomethacin, or desferoxamine improved cerebral metabolism and electrocortical brain activity after asphyxia in newborn lambs, but a combination of the three had no additional effect.[141] In another study, Van Bel et al. showed that allopurinol reduced free radical formation, cerebral perfusion, and electrical brain activity in a group of 11 severely affected infants compared to a comparable control group without significant side effects. [142]

2.3.4.3 Carbon Dioxide and Acidosis

The arterial partial pressure of carbon dioxide ($PaCO_2$) has a potent effect on cerebral perfusion and hypocapnia, which causes vasoconstriction, has been associated with an increased risk of periventricular leukomalacia in ventilated premature infants.[143] Vannucci et al. found that 7-day-old rats with mild hypercapnia ($PaCO_2$ = 54 mm Hg) had less hypoxic-ischemic injury than pups with normocapnia, while hypocapnia was associated with greater injury.[144] This suggests that hypercapnia could be a clinically useful intervention, although this has not been established by clinical trials. However, the observation that respiratory acidosis is not harmful is consistent with other data indicating that acidosis, whether respiratory or metabolic, is not itself directly harmful to the neonatal brain.[145] Although severe lactic acidosis is a marker for hypoxic ischemia severe enough to damage the brain, evidence from Vannucci's laboratory suggests that it is the degree of energy deficiency rather than the lactic acid that causes injury.[145]

2.3.4.4 Glutamate Antagonists

When administered before or within 1–2 hours after experimental hypoxic ischemia, NMDA type glutamate receptor-channel antagonists such as dizocilpine (MK-801), ketamine, or dextromethorphan, are the most potent neuroprotective agents in the neonatal brain.[90–93] In rodents this protection is related to the enhanced toxicity of NMDA receptor overstimulation at 6–7 days from ischemia or trauma, probably related to a special role that these receptors play in development at this age.[94] Non-NMDA glutamate receptor antagonists also have some protective effects in neonatal rodent models of hypoxic ischemia, though less than for NMDA antagonists.[92] Non-NMDA antagonists have recently been reported to protect against white matter injury in a neonatal rodent model, suggesting a potential intervention for periventricular leukomalacia.[61] Although NMDA antagonists are less effective against global ischemia in adult animals, they are strongly protective against hypothermic circulatory arrest in young dogs.[77] Magnesium, which normally blocks the NMDA channel is also a potent protective agent in the immature rodent model, although it was not effective in a model of umbilical cord occlusion in sheep.[145,147] Magnesium therapy for human infants with asphyxia may be limited by its tendency to produce systemic hypotension.[96,148]

2.3.4.5 Nitric Oxide Synthesis Inhibition

Inhibition of nitric oxide synthesis shows promise in animal models of hypoxic ischemia. In the 7-day-old rat model, we found that 7-nitroindazole (7-NI), an inhibitor of the neuronal isoform of NOS, is protective in a dose of 100 mg/kg that is sufficient to suppress nNOS catalytic activity in the brain for 6–9 hours after the insult, but not at a lower dose of 50 mg/kg that reduces nNOS for only an hour. This is consistent with previous reports that nNOS containing neurons are less vulnerable to ischemic injury than other neurons and respond to injury with an increase in immunoreactivity in injured regions of the brain after the insult.[99,149,150] Recent experiments also showed that inhibition of immunologic NOS (iNOS or NOS II) is also protective in the 7-day-old rodent model. Tsuji et al.[151] found that the iNOS inhibitor aminguanidine administered before the insult produced by carotid ligation and exposure to hypoxia and for 3 days afterward markedly reduced the delayed rise in NO metabolites in the ischemic hemisphere, and markedly reduced brain injury. Selective inhibition of NOS isoforms may have promise for protecting the brain, in contrast to some nonspecific inhibitors of NOS which may produce mixed results because of interference with endothelial NOS (NOS III) that regulates cerebral blood flow.[98]

2.3.4.6 Caspase Inhibition

Inhibition of cysteine-containing, aspartate-preferring proteases (caspases) is potentially the most potent neuroprotective pharmacologic strategy in the neonatal brain yet discovered, aside from inhibition of NMDA channels.[152,153] Caspase 3, the main downstream executioner caspase, and others are strongly activated after hypoxic ischemia in the neonatal brain, probably related in part to the prominent role that the

apoptosis-necrosis continuum plays at this age.[152] Caspase inhibition can produce synergism with NMDA antagonism because caspases are activated downstream in the excitatory cascade. Cheng et al. showed that pan-caspase inhibition provides strong neuroprotection in the neonatal rat model of hypoxic ischemia when given more than 2.5 hours after completion of hypoxia.[120] Inhibition of another family of cysteine proteases, the calpains, also has some neuroprotective effect, and these protease families interact with each other following an ischemic insult.[128] Although current caspase-inhibiting peptides have difficulty penetrating the blood brain barrier, this is a potentially exciting new area for therapeutic intervention.

2.3.4.7 Neuronal Growth Factors

Neuronal growth factors such as nerve growth factor, basic fibroblast growth factor (bFGF), insulin-like growth factor I (IGF-I), and brain-derived neuronal growth factor (BDNF) have all been demonstrated to have a neuroprotective effect in neonatal models of hypoxic-ischemic brain injury.[154–160] For example, Hossain et al. showed that implantation of endothelial cells genetically engineered to secrete human FGF (FGF-1) protected against quinolinate, an endogenous NMDA receptor angonist in neonatal rats.[155] The neuroprotective effect of BDNF against injury in hypoxic ischemic neonatal rats is correlated with inhibition of caspase-3 activity, acting through the Ras-MAP kinase signaling pathway.[156,160] This suggests that one important component of the neuroprotective action of growth factors is the ability to activate intracellular signaling cascades that block pathways involved in apoptosis and caspase activation.

2.3.4.8 Glucocorticoids

Glucocorticoids such as dexamethasone have a potent effect on the outcome of brain injury, but this effect is markedly dependent on the timing of administration in relation to the insult. Although administration of corticosteroids immediately before or after the insult has little effect on injury and may increase mortality in rat pups,[96,161] Barks et al. showed that administration 24 hours before was strongly protective. Adminstration 6 hours before also offered some protection.[162] Although the effects of administration at 24 hours before the insult suggest the possibility of an induction of gene expression, the mechanism for this effect remains to be discovered. Recent clinical reports indicate that dexamethasone may have an adverse effect on brain development in premature infants when given after birth to prevent lung disease.[163,164]

2.3.4.9 Hypoxic Preconditioning

Hypoxic or ischemic preconditioning is the reduction in injury to an organ that occurs when it has been exposed to an earlier insult, often at an interval of 24 hours. Gidday et al. reported a preconditioning effect in neonatal rat pups exposed to an 8% oxygen environment for 3 hours at 24 hours prior to an hypoxic ischemic insult.[165] This group also reported that this effect may be mediated by nitric oxide derived

from endothelial NOS.[166] An understanding of the mechanism for this effect could help to uncover new steps in the pathways to mediate hypoxic-ischemic injury.

2.4 CONCLUSION

Ischemic injury in the neonatal brain differs from the adult in several ways, including mechanisms of injury, patterns of selective vulnerability, and the contribution of apoptosis vs. necrosis as a mode of cell death. Many of the molecular mechanisms of injury appear to be similar to those activated in the adult brain, but major variations occur in large part related to the developmental importance of these pathways in brain development at the time of injury. Another important age-related difference is the greater plasticity of the immature brain, which contributes to different patterns of recovery after fetal or neonatal injuries.

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